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**14. ABSTRACT** Stromal-epithelial interactions are important in development and cancer of the prostate. Estrogen receptor functions as a transcription factor to regulate gene expression. One such ER regulated gene is the protease cathepsin D (CathD). *This proposal has two immediate objectives. The first is to determine how overexpression of cyclin D1 (CD1) in the stroma induces the upregulation of the estrogen regulated gene CathD. The second objective is to determine how overexpression of CathD in the stroma can contribute to tumorigenesis in the epithelium.* Results show 1) CD1 interacts with the ER! to modify the expression of estrogen regulated genes like CathD in prostate fibroblasts. 2) ER signaling in the stroma contributing to CAFs induced tumorigenesis in adjacent epithelium. 3) Stromal specific overexpression of CathD promotes prostate tumorigenesis through activation of TGF" signaling pathways.

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**Table of Contents**

**Page**

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## Introduction

Historically, the field of cancer biology has primarily been focused on studying the malignant cells comprising tumors [1]. The emergence of the tumor microenvironment as a field of active study is providing some much needed insight into how non-malignant cells associated with cancer (cancer associated stroma) can promote or suppress tumorigenesis[2]. The stromal phenotype has been shown to be a powerful prognostic indicator of cancer progression and of patient death underlining their importance in defining lethal versus indolent phenotypes [3].

Stromal-epithelial interactions are important in the development and cancer of the prostate [4-6]. During carcinoma evolution, the stroma surrounding the nascent tumor undergoes phenotypic alterations that have been shown to enhance the invasive potential of the epithelial tumor [7-9]. These stromal-epithelial interactions are mediated, in a large part, by paracrine signaling between epithelial tumor cells and neighboring stromal fibroblasts [9]. In addition to receiving signals from malignant epithelial cells, the stromal fibroblasts stimulate tumorigenesis by releasing factors that act on adjacent epithelial tumor cells or exchange enzymes that modify local microenvironment promoting the proliferation and survival of the neoplastic cells [8-11].

Modifications to the local tumor microenvironment are accomplished through the actions of several different families of proteins, including proteases produced by either the tumor or the stroma [12]. These enzyme families include matrix metalloproteases (MMP), cysteine, and serine proteases, which have been shown to play a role in the degradation of the basement matrix, promotion of angiogenesis, and the liberation of growth factors to stimulate tumor cell growth [13 14].

Cathepsin D (CathD) is a ubiquitous lysosomal aspartic endoproteinase, CathD, has been shown to be involved in a number of physiological processes. CathD is known to play a critical role in barrier function, regulation of apoptosis, and epithelial differentiation [15-17]. In cancer however, CathD is overexpressed and hypersecreted in various malignancies including PCa [18 19]. In breast cancer, CathD expression is associated with a poor prognosis and increased likelihood for the development of metastasis [20]. Experimental evidence has shown CathD can stimulate the proliferation of PCa cell lines [21].

There are limited data defining CathD's function in prostate cancer progression. Some studies have concluded that CathD is overexpressed in the epithelium and stroma of PCa, and may promote proliferation [21,22]. Other studies have concluded that CathD produced by PCa may be inhibiting tumor growth [23,24]. Despite all the advances in basic and translational research the mechanisms underlying the development and progression of cancer to this date are still poorly understood.

Androgens and the androgen receptor have been shown to have an integral role in mediating proliferation, differentiation, and maintenance of the prostate epithelium [25]. Not to be overshadowed by the role androgens play in PCa, estrogens also play a profound role in prostate carcinogenesis [26].

Estrogens act indirectly on the prostate gland by suppressing the release of leuteinizing and follicle stimulating hormones from the pituitary gland, a form of chemical castration, which prevents the production of testosterone by the testis. The lack of androgenic signaling in the prostatic stroma induces apoptosis in the adjacent epithelium. Exogenously supplied estrogens also act directly on the prostate mediated by the estrogen receptor in the stroma to induce squamous metaplasia (SQM) in the epithelium [27]. Work, performed 69 years ago by Huggins and Hodges, showed the profound effects estrogens have on the prostate [27]. In other animal models, intermittent exposure to estrogens during neonatal and/or perinatal development induces dysplastic 5 changes in the adult prostates of mice [28,29]. Prolonged exposure to estrogens in combination with androgen in the NBL rat model produced a 100% incidence of PCa. Treatment with androgen alone induced PCa with a 40% incidence [30].

The actions of estrogen are mediated through two receptor subtypes estrogen receptor-alpha (ERα) and estrogen receptor-beta (ERβ). Expression of ERα in the prostate is localized to the stroma and becomes elevated during the progression of PCa. The expression of ERβ in the prostate is localized to the epithelium and expression is lost during PCa progression [31]. The estrogen receptor family function as transcription factors and regulate the expression of a number of different genes. One such ER-regulated gene is the aspartic endopeptidase cathepsin D (CathD) [32,33]. CathD is known to be involved in a number of physiological processes as well as in the regulation of apoptosis [34,35]. In various malignancies i.e. breast, and colon cancers CathD is overexpressed and hypersecreted [36] [37]. Treatment of breast cancer cell lines with synthetic peptides corresponding to CathD induced increased expression of anti-apoptotic genes and cell cycle regulators [34]. CathD is hypothesized to bind the mannose-6-phosphate receptor (M6PR)/insulin-like growth factor II receptor (IGFIIR) [21]. However, blocking of the IGFIIR did not abrogate the pro-mitogenic effects of CathD [38].

Our lab has previously published on several molecules found to be aberrantly expressed in cancer associated fibroblast (CAFs) (including cyclin D1 (CD1), and stromal derived factor-1 (SDF-1) that induce tumorigenesis and malignant transformation in tissue recombination experiments [9,39]. Unpublished findings comparing CAF primary CD1-overexpressing-normal primary fibroblast ($NPF^{cyclin\ D1}$) and parental NPF revealed that the expression of CathD was overexpressed 7-fold in both CAF and $NPF^{cyclin\ D1}$ in comparison to NPF. The cell cycle regulator CD1 and ERα are known to interact and can induce estrogenic gene transcription [40]. This suggests that the overexpression of CathD in PCa associated stroma is due to the interaction of ERα and CD1. ***This proposal has two objectives. The first is to determine how overexpression of CD1 in the stroma induces the upregulation of the estrogen regulated gene CathD. The second objective is to determine how overexpression of CathD in the stroma can contribute to tumorigenesis in the epithelium.*** We believe that changes in the stroma result in alterations in stromal-to-epithelial paracrine signaling. This altered environment promotes the initiation and progression of tumorigenesis.

**Body**

The original stated goal in the approved statement of work for Task 1 was to determine if CD1 over expression modifies estrogen regulated genes through interaction with endogenous ERα in prostate fibroblasts. To accomplish this task we developed a benign human prostate stromal cell line BHPrS to co-express CD1 and ERα to use as tool for examining the interaction of CD1 with ERα on the CathD promoter. In figure 1A, Co-immunoprecipitation experiments were performed to determine if CD1 could interact with ERα in prostate stromal cells. We reported in our last progress update, the co-overexpressing $BHPrS^{CD1\text{-}ER\alpha}$ cells demonstrate CD1 and ERα can interact when ectopically expressed. Overexpression of CD1 in BHPrS alone demonstrated interaction with endogenous ERα in prostate stromal cells. We next examined if CD1 overexpression modifies ERα transcriptional activity. Luciferase activity assays were performed with the use of estrogen responsive element fused with the luciferase gene. These experiments were performed in presence/absence of β-estradiol. In comparison to the empty vector control $BHPrS^{EV}$, CD1 overexpression induced increased ER transcriptional activity in the absence of hormone. This indicates that CD1 can induce the transcriptional activity of the ER with out the ligand bound in prostate stromal cells. This finding is in agreement with studies of ER and CD1 in breast cancer [40]. Co-overexpression of ERα and CD1 in BHPrS cells induced greater luciferase expression compared to CD1 overexpression alone, both in the presence and absence of hormone. These data show that CD1 can interact with ERα to drive transcriptional activity of the estrogen receptor on non-chromosomal DNA. To determine if the CD1-ERα interaction binds chromosomal DNA we performed chromatin immunoprecipitation (ChIP) experiments with the $BHPrS^{CD1}$ and $BHPrS^{CD1\text{-}ER\alpha}$ cell lines. Overexpression of CD1 in BHPrS showed greater than 11 fold recruitment over the IgG control of CD1 to the estrogen receptor

element (ERE) in the CathD gene in the absence of hormone. In the presence of hormone CD1 recruitment to the ERE in the CathD gene was only increased 8 fold over the IgG control.

We have previously shown that CD1 overexpression in benign prostate fibroblast cells ($NPF^{CD1}$) produces a phenotype similar cancer associated fibroblasts [39]. A comparison of gene expression profiles from $NPF^{CD1}$ and CAFs cells identified CathD to be overexpressed in both cell types in comparison to NPFs. Task 1 from the approved statement of work sought to to determine if CD1 over expression modifies estrogen regulated genes through interaction with endogenous ERα in prostate fibroblasts. Our experimental approach showed that CD1 overexpression leads to aberrant ERα activity on the CathD gene.

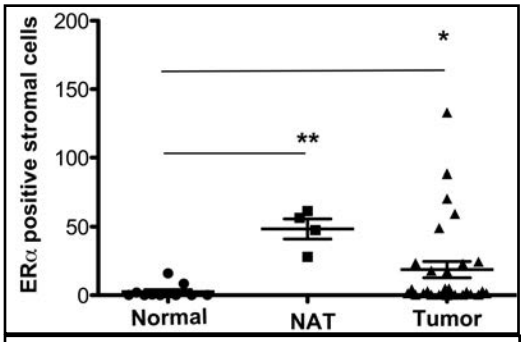


**Figure 1. ERα is overexpressed in the stroma of malignant prostate tissue.** Quantitation of total ERα expressing stromal cells in each prostate core. Statistical analysis performed by ANOVA, *p-value≤ 0.05, **p-value≤ 0.0005.

Our original stated goal in the approved statement of work for Task 2 was to determine if inhibition of the ERα in signaling in the stroma abrogates the ability of CAF and/or $NPF^{cyclin\ D1}$ to induce tumorigenesis. Our rational for this task is based on the understanding of the hormone levels in aging men. Levels of circulating testosterone in younger men are greater in comparison levels of estrogen. After 30 years of age, the hormone ratios in men start to change, with older men having lower levels of circulating testosterone and greater levels of estrogen [41]. Knockout animal models have been used to examine the role of the estrogen receptor in prostate. Recombination experiments with ERβ knockout mesenchyme with wild type epithelium produced squamous metaplasia (SQM) when supplemented with a synthetic estrogen, however, SQM was not observed in ERα knockout mesenchyme was combined with wild type epithelium, indicating the importance of ERα in the stroma [42].

In figure 1, we examined the expression of ERα in human prostate clinical samples using a tissue microarray which contained 30 cases of adenocarcinoma, 5 cases of normal prostate tissue, and 5 cases of normal prostate tissue adjacent to malignant tissue (NAT). The tissue array contained duplicate cores per case. Quantification of nuclear ERα in stro-mal regions was significantly greater in malignant prostate tissue in comparison to normal prostate tissue.

In figure 2A, and 2B, gene expression analysis of ERα and aromatase in primary fibroblasts isolated from NPF and CAFs showed significantly greater expression in CAFs in comparison to NPFs with a greater than 5 fold difference. Due to our observations of increased ERα expression in CAFs and in malignant human tissue, we questioned the role of the ER in the cancer associated stroma's ability to promote transformation in tissue recombination experiments.

To accomplish our approved second task we stated CAF and $NPF^{CD1}$cells would be engineered with shRNA specific to ERα. We attempted to transduce our different fibroblast cells with the shRNA constructs to make stable cell lines, however, CAFs and $NPF^{CD1}$ cells under went replicative senescence. A downside to working with non-immortalized cells, is the limitation to the number of cell cycle replications. To overcome this pitfall and still address the second task we decided to use a pharmacologic approach and inhibit the ER

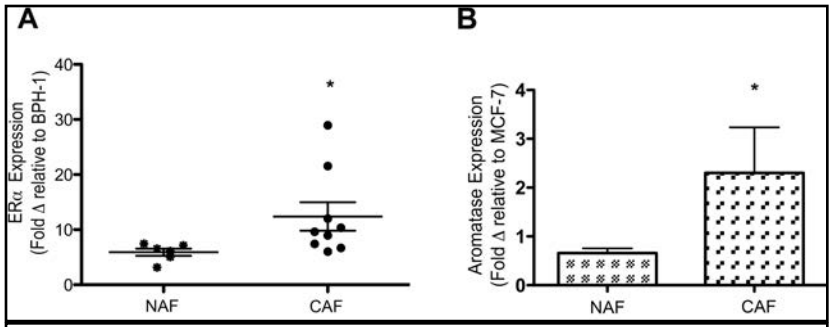


**Figure 2. ERα and Aromatase expression is upregulated in patient derived CAFs**. Real-time expression analysis of (A) ERα, and (B) Aromatase, in patient derived NAFs and CAFs. Results presented as fold change relative to BPH-1 cells and MCF-7 cells

in signaling with the use of Tamoxifen. We know that the addition of testosterone is sufficient to induce a malignant conversion in recombination experiments of CAFs with BPH-1 cells[26]. Inhibition of the ER with tamoxifen was sufficient at preventing the ability of CAFs to induce a malignant conversion in the adjacent epithelium in our model system (Figure 3).

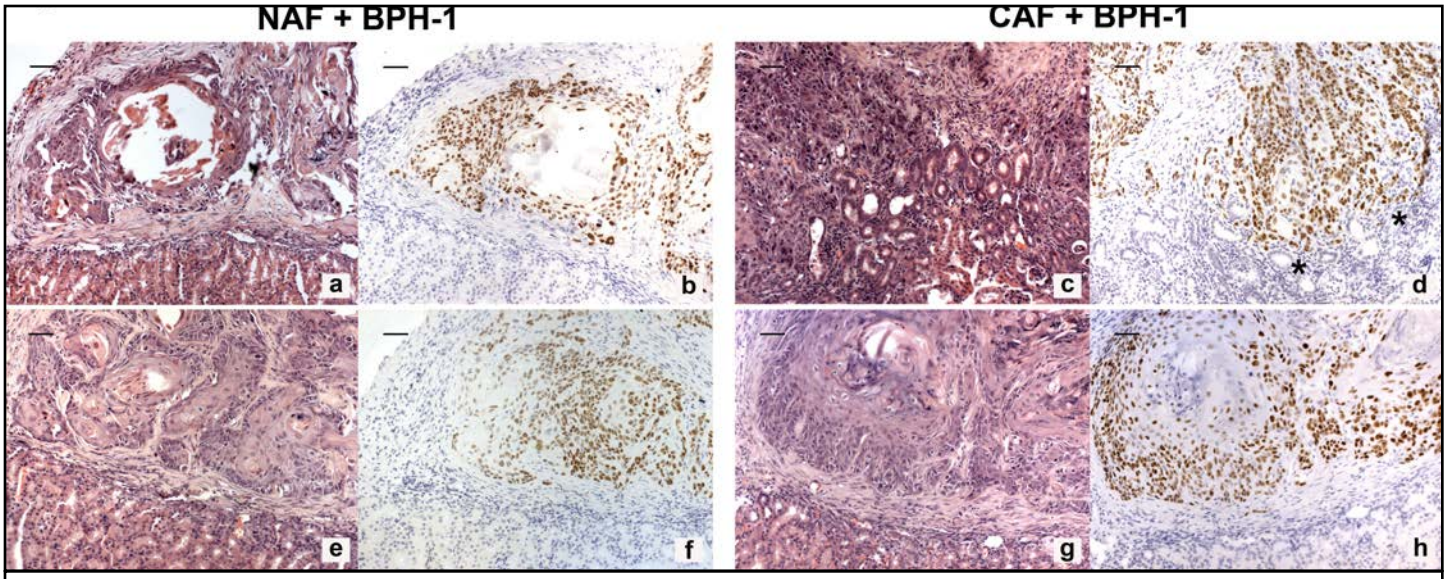


**Figure 3. Tamoxifen abolishes CAF induced transformation of adjacent epithelium.** Tissue recombinations of BPH-1 cells with NAFs (left) or CAFs (right). Mice supplemented with testosterone (a,b,c,d) or testosterone and tamoxifen (e,f,g,h). Invasion of BPH-1 into kidney denoted by asterisk (f).

In figure 4 we quantitated the distance the transformed BPH-1 cells invaded into the kidney of the mouse. A comparison of BPH-1 cell invasion into the kidney in recombinations with CAFs shows a significant decrease in mice treated with tamoxifen. These results suggest that the activation of the ER in the stroma plays a critical role in prostate tumor progression. From our knowledge of the biosynthesis of sex steroid hormones, we know that testosterone can be directly converted in to estrogen by the aromatase enzyme. The co-administration of Testosterone (T) with Estrogen (E) stimulates cancer progression and malignant transformation in tissue recombination experiments of rat UGM (rUGM) and BPH-1, however, the single administration of T alone induces benign growth [26,43]. In the majority of models for hormonal carcinogenesis, estrogen along with the actions of the ER are required for maximal carcinogenic response to androgens. Recombinations of NPF + BPH-1 in mice supplemented with T produced benign growths with no malignant conversions. CAF + BPH-1 in mice supplemented with T induced a malignant conversions denoted by the BPH-1 cells invading into the mouse kidney. Recombinations of NPF +

BPH-1 in mice supplemented with T + E resulted in histologies resembling CAF + BPH-1 recombinations. The addition of estrogen in combination with T, drove a malignant conversion in the adjacent epithelium. This result is similar to published findings of tissue recombinations experiments of rUGM with BPH-1.

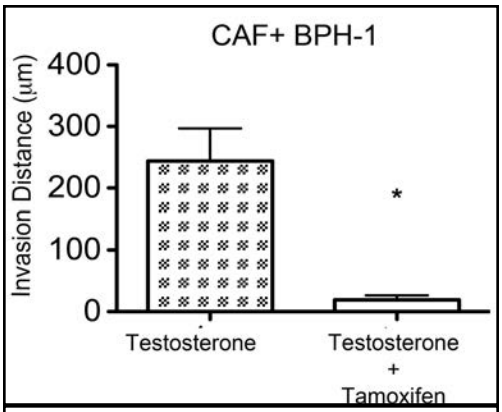


**Figure 4. Inhibition of stromal ER abrogates BPH-1 invasion.** Measurement of invasion distance in recombinations of BPH-1 cells with CAFs in mice supplemented with testosterone or testosterone and tamoxifen. Distance measured in micrometers. Significance determined by t-test, p-value $\leq 0.05$.

| Results of Tissue Recombinations | | | | |
|---|---|---|---|---|
| Treatments | rUGM | EV | ERα | ERβ |
| NT | No Growth | No Growth | No Growth | No Growth |
| T | Small Benign Growth | Small Benign Growth | Small Benign Growth | Small Benign Growth |
| T + E | Largest Malignant Tumors | Small Benign Growth | Large Malignant Tumors | Small Benign Growth |
| E | Small Growth SQM | Small Growth SQM | Small Growth SQM | Small Growth SQM |

**Table 1**. Results of various recombinations on BPH-1 cells with rUGM, BHPrSEV (**EV**), BHPrSERα (**ER**α), and BHPrSERβ (**ERβ**), supplemented with the different hormones testosterone (**T**), estrogen (**E**), and testosterone + estrogen (**T+E**).

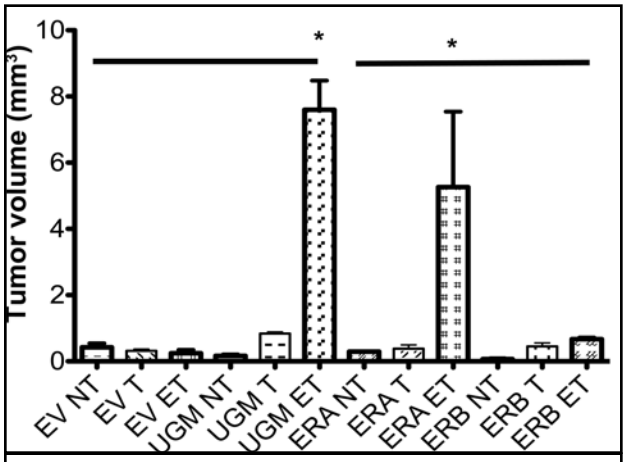


**Figure 5. Overexpression of ERα in prostate stromal cells induces tumorigenesis in vivo.** Comparison of tumor volume in recombination of BPH-1 cells with BHPrSEV (EV), BHPrSERα (ERA), BHPrSERβ (ERB), or rUGM. Castrated mice were supplemented with testosterone (T), estrogen and testosterone (ET), or no treatment (NT). Significance determined by ANOVA, p-value ≤ 0.05.

Since we observed increased expression of ERα in the stroma of malignant prostate tissue, we examined the consequences of ERα modulation in stromal cells. To address this question we engineered BHPrS cells to stably overexpress ERα (BHPrSERα). As a control, we also overexpressed ERβ in BHPrS (BHPrSERβ ). We prepared tissue recombinations using the prostate epithelial cell line BPH-1 as a reporter with the BHPrSERα and BHPrSERβ stromal lines and grafted under the renal capsule in castrated SCID mice supplemented with a combination of 17β-estradiol and testosterone pellets, testosterone alone, or no-treatment (NT). As a control we also used rUGM recombined with BPH-1 cells and grafted under the renal capsule in castrated mice supplemented pelleted testosterone and 17β-estradiol. Recombinations of rUGM under these conditions induces carcinogenesis of the adjacent BPH-1 cells as previously described by Wang et al. 2001[26]. Table 1 shows the results from the various recombinations with different hormone treatment groups. After an 8 week engraftment period, recombinations of BHPrSERα+ BHP-1 cells gave rise to significantly larger tumors in comparison to recombinations of BPH-1 with either BHPrSERβ or BHPrSEV. Recombinants composed of rUGM + BPH-1 cells grafted in mice supplemented with the same hormone combination produced significantly larger tumors in comparison to castrated mice (Figure 5). Similar experiments were performed in mice supplemented with testosterone and β-estradiol independently. These resulted in smaller growths in comparison to mice supplemented with the combination of hormones.

Our study highlights human ERα expression in the cancer associated stroma. The overexpression of the ERα in benign human prostate stromal cells promotes the expression of markers associated with the reactive stromal phenotype. We see that overexpression of ERα specifically in the stroma is sufficient to drive tumorigenesis and induce the malignant conversion of initiated, but non-transformed prostate epithelial cells. We also show CAFs express relatively higher levels of ERα and the enzyme aromatase in comparison to NAFs. We believe the increased expression of aromatase aids in producing a local estrogen rich environment promoting the development of prostate tumorigenesis. Lastly this second task identifies stromal ERα as a target for therapeutic intervention by showing pharmacologic inhibition of the ER in the cancer associated stroma inhibits malignant transformation in the adjacent epithelium.

Our final approved task was to determine how stromal production of CathD promotes tumorigenesis. The overexpression of CathD in neoplastic cells and neoplastic associated connective tissue was described close to 30 years ago, and is reported to play several roles in cancer progression [44,45] [46,47]. To accomplish this

task we engineered BHPrS cells to overexpress CathD (BHPrS$^{CathD}$) to perform *in vitro* and *in vivo* experiments. Sub-renal capsule xenograft experiments were performed using the BHPrS$^{CathD}$ recombined with BPH-1 cells. As we previously reported, after an 8 week engraftment period, overexpression of CathD in the stroma induced a malignant transformation in the adjacent epithelium along with significantly larger tumors in comparison to recombinations with the empty vector control BHPrS cells. The reverse experiment was performed using BPH-1 cells made to overexpress CathD (BPH-1$^{CathD}$) and recombined with parental BHPrS cells and grafted under the kidney capsule. After a period of 8 weeks, epithelial overexpression of CathD failed to induce significant differences in growth or a malignant transformation. Our model identifies that stromal specific expression of CathD plays a role in promoting tumorigenesis. Our model is further supported by our findings of CathD knockdown expression in CAFs abolishes the ability to induce a malignant transformation in adjacent epithelium.

To delve deeper into understanding the role of stroma specific expression of CathD promoting tumorigenesis, we examined growth factor signaling pathways in the BPH-1 cells treated with conditioned media from BHPrS$^{CathD}$. Conditioned media collected from BHPrS$^{CathD}$ and BHPrS$^{EV}$ was added to monolayer cultures of BPH-1 cells prior to isolation of protein lysates. Western blot analysis was performed on BPH-1 cell lysates for changes in the growth factor signaling pathways MAPK, and Akt/PKB. No differences were observed in the phosphorylation states of MAP kinases p38 of ERK1/2, however, we did observe increased p-Akt levels in BPH-1 cells supplemented with BHPrS$^{CathD}$ conditioned medium.

Our laboratory has previously published a study identifying a possible mechanism for CAFs can induce tumorigenesis in the adjacent epithelium. The study identified two molecules, transforming growth factor-beta (TGF-β) and stromal cell derived factor-1 (SDF-1) as being overexpressed in CAF cells. These factors were acting in a paracrine manor on the epithelial cells resulting Akt hyperactivation [9]. We questioned whether the increased levels of p-Akt seen in our experiments with the conditioned media isolated from BHPrS$^{CathD}$ cells was related to TGF-β activity. It has been previously shown in *in vitro* experiments that CathD can liberate TGF-β from the latency inhibitor complex, allowing for activation of the TGFβ receptor (TGFBR) complex [48]. In vivo experiments using the CathD prozyme, showed CathD can be fully activated in the extracellular environment and cleave substrates in the mouse prostate[49]. We examined our tissue recombinations of BHPrS$^{CathD}$ with BPH-1 cells for increased TGF-β signaling. Immunohistochemical staining for p-SMAD2/3, the immediate downstream substrate of TGFBR activation, showed significantly greater p-SMAD2/3 staining in CathD overexpressing recombinants. We also examined differences in the TGF-β responsive gene collagen type 4 α2 (ColIVα2). Staining for showed increased deposition of ColIVα2 in recombinations overexpressing CathD. Masson's trichrome staining of tissue xenografts from the CathD overexpressing stromal cells also revealed increased production of collagen in comparison to recombinations with the EV control stromal cells. Collectively we concluded that stromal derived CathD was promoting tumorigenesis through the activation of TGF-β signaling pathways.

The second line item under the approved third task was to determine if the conversion of pro-CathD to the active protease is dependent on the presence of glycosaminoglycans (GAGs) on the surface of PCa cells. To accomplish this task we treated a series of PCa cell lines with heparinase to remove heparin sulfate containing proteoglycans from the cell surface prior to the addition of pro-CathD. The heparin sulfate proteoglycans have been previously shown to convert the CathD zymogen to the active state[48]. Completion of the CathD activity assay after treatment with heparin lyase did not show any inhibition in the conversion of pro-CathD to the active protease. We also performed this experiment with 5-(N-ethyl-N-isopropyl)-amiloride (EIPA), a specific inhibitor for Na(+)/H(+) antiporters. It has been shown that human PCa and PCa cell lines have lower extracellular pH due to increased proton pump expression [50]. We hypothesized that CathD activity was dependent on the activity of proton pumps present on the surface of PCa cells. PCa cell lines treated with EIPA show drastic decreases in extracellular CathD activity similar to levels when of cell lines treated with the CathD

inhibitor pepstatin A. Our results show that extracellular CathD activity is not dependent on heperan sulfate containing proteoglycans, however is dependent on the extracellular pH.

In summary the aims executed in this grant provided advances in the field of PCa biology by 1) identifying expansion of ERα positive cells in PCa reactive stroma and in patient derived primary CAFs. 2) Showing pharmacological inhibition of the ERα abrogates epithelial malignant conversion by the cancer associated stroma. 3) Showing that ER responsive genes, i.e. CathD, are expressed in the reactive stroma of malignant prostate tissue and promotes prostate tumorigenesis. We have also identified a possible mechanism to explain how stromal derived CathD overexpression can promote tumorigenesis through TGF-β signaling. These data further demonstrate stromal ERα role in prostate tumorigenesis and highlight ERα as a therapeutic target for chemoprevention.

**Task 1. To determine if CD1 over expression modifies estrogen regulated genes through interaction with endogenous ERα in prostate fibroblast:**

1. Develop BHPrS cells that co-express CD1 and ERα (Months 1-3). **Completed**
2. Characterize BHPrs$^{ERα\text{-}CD1}$ stable cell lines (Months 4-6). **Completed**
    a. Determine expression of CD1 and ERα by western blot.
    b. Quantify proliferation by crystal violet assay.
    c. Quantify luciferase expression after transient transfection with luciferase reporter construct fused with an estrogen responsive element.
3. Perform biochemical studies examining CD1 and ERα physical interaction on CathD promoter (Months 7-12). **Completed**
    a. ChIP assays

**Task 2. To determine if inhibition of the ERα signaling in the stroma abrogates CAF and/or NPF$^{cyclin\ D1}$ ability to induce tumorigenesis:** Completed with approved modifications described below.

1. Abrogate stromal ER activity by pharmalogical inhibition with tamoxifen. **Completed**
    a. Perform tissue recombinations of BPH-1 cells with CAFs and NAFs grafted in castrated SCID mice supplemented with testosterone, or testosterone + tamoxifen pellets.
    b. Determine the effects of ER inhibition by histology.
2. Determine expression levels of ERα in human clinical prostate specimens. **Completed**
    a. Quantitate IHC staining for ERα in a prostate tissue microarray.
    b. Measure expression levels of ER and estrogen producing enzymes in patient derived NAFs and CAFs.
3. Engineer ERα overexpressing BHPrS cells and perform xenograft studies using BHPrSERα recombined with BPH-1 cells. **Ongoing**
    a. Evaluation of tumorigenesis by measuring tumor volume.
    b. Evaluation of malignancy and invasion by IHC.

**Task 3. To determine how stromal production of CathD promotes tumorigenesis:**

1. Characterize the effects of conditioned media from BHPrSCathD on BPH-1 and BHPrE1 cell lines (Months 20-24). **Completed**
    a. Quantify CathD induced proliferation of epithelial cell lines.

b. Examine cell signaling pathways i.e. phospho-Akt, phospho-p38 MAPK by western blot analysis.

2. Examine the conversion of pro-CathD (inactive) to CathD (active) by GAG present on the surface of PCa cell lines (Months 22-36). **Ongoing**
   a. Perform activity assays using purified pro-CathD added to the PCa cell lines LNCaP, C4-2B, and PC3.
   b. Perform activity assays for CathD using the BHPrSCathD conditioned media added to PCa cell lines.
   c. Perform CathD activity assays using purified pro-CathD and BHPrSCathD conditioned media after protein glycosylation is inhibited using soluble inhibitor.

**Task 4. Prepare manuscript(s) for publications.** One Completed, another in preparation.

**Key research accomplishments**

- Prostate stromal cell lines were generated and characterized.
- ERα is overexpressed in the cancer associated stroma and in normal associated stroma (**figure 1**).
- Patient derived CAFs display increased ability for the conversion of testosterone to estrogen, along with increased ER gene expression. **(figure 2)**.
- Pharmacological inhibition of stroma ER with tamoxifen inhibits CAF induced tumorigenesis and malignant transformation *in vivo* **(figures 3 and 4**).
- Overexpression of ERα in benign stromal cells induces robust tumorigenesis and conversion to malignancy in adjacent epithelial cells in tissue recombination experiments (**figure 5 and table 1**).

**Reportable outcomes**

The following publication was referenced by this training grant during the last year.

1. **Pruitt, FL**., He, Y., Franco, OE., Jiang, M., Cates, JM., Hayward, SW. Cathepsin D acts as an essential mediator to promote malignancy of benign prostatic epithelium. The Prostate. 2013Apr;73(5):476-88.

**Conclusions**

Significant progress has been made towards achieving the stated goals considering the technical limitations experienced in the second aim. This work has produced one publication along with a second publication in the works.

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